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Chapter 37

Progress in sexual coral reproduction at Oceanopolis

Dominique Barthelemy

Oceanopolis, Port de Plaisance du Moulin Blanc, 29200 Brest, France
dominique.barthelemy@oceanopolis.com

ABSTRACT

Using the techniques developed within SECORE (SExual COral REproduction, a network of public aquariums and research institutions, which aims to develop sexual coral reproduction in captivity) has enabled us to obtain planulae from nine scleractinian species at Oceanopolis:
Favia fragum, Pocillopora damicornis, P. verrucosa, Stylophora pistillata, Seriatopora hystrix, Porites sp.*, Euphyllia paradivisa, Stylocoeniella guentheri* and *Pavona cactus.*
All these species, except for *Stylocoeniella guentheri* and *Pavona cactus*, were already known to belong to the reproductive group of the brooders (internal fertilisation and release of settlement-competent larvae).
Studies are carried out to monitor the planula production cycles for those species, as well as their settlement process and early development of primary polyps.

INTRODUCTION

Scleractinian corals have developed two main modes of sexual reproduction: broadcast spawning (external fertilization, planktonic embryogenesis), and brooding (internal fertilization, release of settlement competent larvae called planulae).
The reproduction of corals by gathering planulae released by brooding species is a new way of breeding these animals in public aquariums. This technique may prove to be particularly useful in reproducing species where propagation by fragmentation is proving tricky or impossible.
Discovering the different parameters that influence the production of planulae and their conditions of settlement may also help us to stimulate their spontaneous development in our tanks. This is one of the goals of SECORE (SExual COral REproduction), a network of public aquariums and research institutions, which aims to develop sexual coral reproduction in captivity.
Captive breeding of the Caribbean brooders *Favia fragum* and *Agaricia humilis* has been well studied and documented (Petersen *et al.*, 2007a). Several generations of *F. fragum* have been bred in captivity and disseminated within the public aquariums community.
Very few other species are currently bred in captivity. Spontaneous appearance of new colonies on the walls or decors of the tanks are occasionally observed in public aquariums, but, most of the time, reproduction is not manipulated by the aquarium staff (Petersen *et al*., 2007b). Among these, the Indo-Pacific species *Pocillopora damicornis* seem to have opportunistic reproduction behaviour. At Oceanopolis, some tanks are now crowded by several generations of spontaneous colonies of *P. damicornis.* Reproduction of this species was then manipulated to reveal planulation cycles (Chaillé de Néré *et al.*, in press).
It might be possible that planulae are released by other brooding species in our aquariums, without finding the right conditions to settle and develop into colonies.
The participation of Oceanopolis in the various activities of SECORE has encouraged us to study in our aquariums the reproduction of different species.

MATERIAL AND METHODS

Species selection

So far, trials have been carried out on nine scleractinian coral species. Most of the selected species are known to be brooders, which justified this choice. The other species

were chosen because they belong to a family in which some species are brooders. The spontaneous appearance of colonies on the walls or decor of some of our aquariums also encouraged us to study these species.
For the experiments only colonies were chosen with a maximum diameter of 15 cm, so as to be able to manipulate them easily and insert them into the planulae collectors.
Due to the time required for manipulation and limited working space, only one colony per species was sampled, the main goal of this study being to select species for further investigations.

Selected species are the following: *Favia fragum, Pocillopora damicornis, P. verrucosa, Stylophora pistillata, Seriatopora hystrix, Porites* sp*., Euphyllia paradivisa, Stylocoeniella guentheri* and *Pavona cactus.* All these species, except for *Stylocoeniella guentheri* and *Pavona cactus*, were already known to belong to the reproductive group of the brooders

AQUARIUM SYSTEM

The corals studied are maintained in an aquarium measuring 1 m x 1 m x 0.50 m (400 L), being one of a set of seven aquariums of the same size. The total volume of this set of tanks is 3,000 L. The bottom of each aquarium is covered with 10 cm of sand, spread on a grid. The life support system is common to all seven tanks. The filter is composed of a simple settling tank and a biological filter. The water is then sterilised by ultra-violet radiation at 25 $mJ.cm^{-2}$. The recirculation rate is approximately 100 % per hour. The water in the set of tanks is continually replaced at 1 % per hour by natural seawater pumped out of the Brest estuary, filtered, sterilised and thermo-regulated.
The temperature of the water is maintained all year round at 26 °C, and the salinity varies between 34 and 36 ‰.
The tanks are lit by 400 W metal halide lamps, with a colour temperature varying from 6,000 to 20,000 K. The tank containing corals of this experiment is lit by 10,000 K lamps (Luxar® MH-T 400 W). Illumination period is 12 hours daily for all those tanks. These aquariums also have natural overhead lighting from skylights in the roof of the building. Photosynthetic active radiance (PAR) measured at the level of corals is between 200 and 400 $\mu E.m^{-2}.s^{-1}$ (LICOR LI-1400 data logger, connected to a LI-193SA spherical sensor). However, because of the natural overhead lighting, peaks of intensity up to 800 $\mu E.m^{-2}.s^{-1}$ have been observed in summer.

PLANULAE COLLECTORS

During the experiment, the colonies studied are placed on a PVC screen located 15 cm below the surface. Each day, in late afternoon, each colony is placed in a perforated PVC cylinder covered with a 200 µm plankton mesh, emerging slightly

Table 1: Studied species

Species	**Family**	**Origin / Year of acquisition**	**Characteristics of the colony Size L x W x H (in mm)**
Euphyllia paradivisa	Euphyllidae	Public aquarium / 2002	4 adult polyps from a mother colony
Favia fragum	Faviidae	Public aquarium / 2005	captive bred F2 generation 35 x 30 x 15
Pavona cactus	Agariciidae	Trade / 2000	90 x 90 x 70
Pocillopora damicornis	Pocilloporidae	Trade / 2000	F1 generation 140 x 110 x 80
Pocillopora verrucosa	Pocilloporidae	Trade / 2000	100 x 65 x 55
Porites sp.	Poritidae	Public aquarium / 2002	90 x 65 x 75
Seriatopora hystrix	Pocilloporidae	Trade / 2000	110 x 80 x 120
Stylocoeniella guentheri	Astrocoeniidae	Trade / 2004	encrusting - 130 x 110
Stylophora pistillata	Pocilloporidae	Trade / 1999	110 x 100 x 95

from the surface. The size of each cylinder is suited to the diameter of the colony.

Before placing the colony in it, the cylinder is immerged by passing the water through the plankton mesh. This detail is important because it ensures that any planulae in the water are not introduced into the trap. Likewise the base of the colonies is regularly inspected and cleaned to avoid the presence of organisms other than the coral under study in the collector. The bottom of each collector consists of a white plastic base which enables the planulae to be easily seen. A slight current in the tank allows the circulation of water through the collectors during the night. In the morning each collector is inspected and any planulae visible to the naked eye are collected with a pipette. Depending on the species, they were found on the walls, in the open water, on the surface or on the bottom. Since some larvae settle rapidly, it is important to take action as early as possible in the morning.
Having removed the colony, the collector is gently raised, leaving only one or two centimetres of water inside. Then a pipette is used to suck up water from near the bottom, to retrieve any living matter that may remain. The reason for this is that in certain species, the embryos or larvae released are almost invisible to the naked eye. The water collected is then inspected through a binocular magnifier.
The planulae are counted, and transferred to a small plastic flask containing a little seawater and a settlement substrate – a ceramic tile developed as part of SECORE (Petersen *et al.*, 2005) – or a fragment of rock colonised by calcareous algae. Between one and four planulae depending on the circumstances are deposited in each flask. The flasks are then kept in a room heated to 24 °C, in natural light at an intensity of 50 $\mu E.m^{-2}.s^{-1}$. The water in the flasks is renewed daily. When the larvae are fixed, the tiles are placed on grids in another tank of the same set. This tank does not contain any known brooding coral species, in order to avoid settlement of unexpected larvae on the tiles. The intensity of light at the level of the tiles is adjusted to 100 - 200 $\mu E.m^{-2}.s^{-1}$.

Figure 1: Planulae collectors

RESULTS AND DISCUSSION

Three types of propagule have been observed in the different species studied:

- Planulae capable of moving actively and settlement competent, generally measuring between 700 and 2,300 µm. This size may depend on the species, but variations are also related to their great ability to change shape.
- Early planula stages, spherical in shape and rotating. These larvae have a diameter of between 150 and 500 µm. In a few rare cases, some of these larvae evolved in a matter of days, became lengthened, mobile and settlement-competent. Such propagules and their development into settlement-competent planulae have already been observed on *Agaricia humilis* (Petersen and Van Moorsel, 2005).
- Embryos or pre-planulae, barely mobile, with a diameter of about 200 µm.

Here, we will only deal with the planula and early planula stages.
This study started on Jan. 25, 2007. The results presented here start from this date until Apr. 10, 2007.

Favia fragum

The parent colonies came from the Rotterdam Zoo where the reproduction of this species in captivity has been studied (Petersen, 2005). The colonies produced from these specimens at Océanopolis are now the third generation reared in captivity. The larvae of this species were released principally between the 8th and the 13th day after the new moon settle on the ceramic tiles in 24 to 48 hours. The peak of planulae release is very similar to the observations of Petersen (2005) on captive raised population (peak 10-13 days after new moon). It can also be compared to the planulating pattern observed by Szmant-Froehlich *et al.* (1985) on *F. fragum* field populations in Puerto-Rico (peak 9-11 days after new moon).

Pocillopora damicornis

Our attention was drawn to this species after the spontaneous appearance of numerous colonies on the walls or decor of some tanks. This fast-growing species seems to be particularly opportunist and is therefore a good subject for studying sexual reproduction. The parent colony used is from the F1 generation. The production of planulae in this species is closely correlated with the cycles of the moon, with peaks of release between the fourth and the twelfth day after the new moon. Between June and August 2006, when four colonies were being studied simultaneously, in all cases the peak release for each of the colonies occurred on the thirteenth day after the new moon (Chaillé de Néré *et al*., 2006). The planulae of *P. damicornis* are very mobile and show a high density of zooxanthellae. Their size is variable because of their ability to change shape, but their average length is 2 mm. They generally metamorphose and settle within 24 hours after their release. They seem able to settle on very varied substrates, with or without bio-film, which

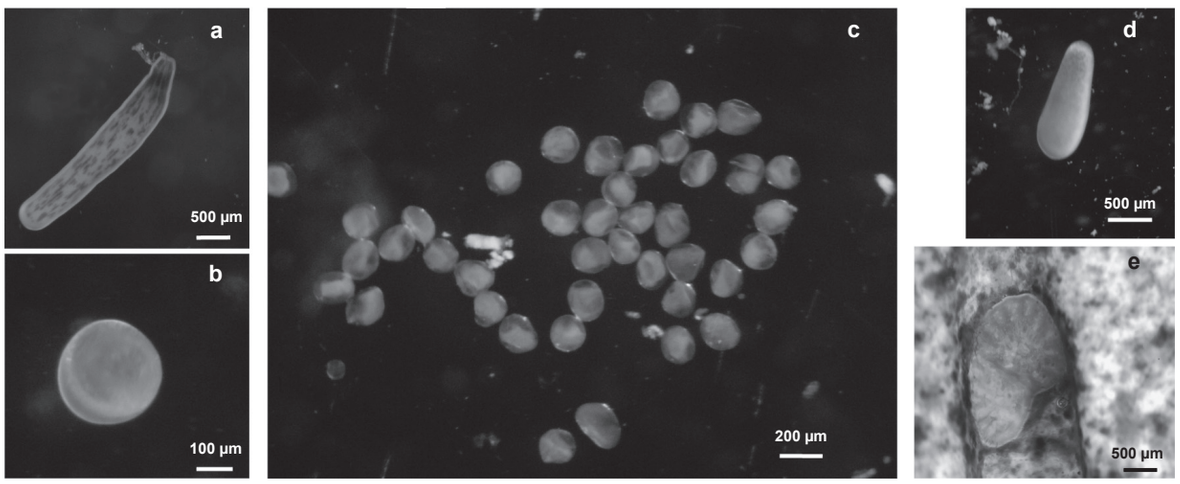

Figure 2:
2a: Stylocoeniella guentheri: *planula, 2b: early planula stage,*
2c: Euphyllia paradivisa: *embryos,*
2d: Favia fragum*: planula, 2e: 22 days. 2 fused primary polyps.*

explains the opportunistic nature of this species and its spontaneous reproduction in the tanks.

Pocillopora verrucosa

Seventeen planulae were produced by this colony during the period of study, without it being possible to identify a release cycle. The mobility and size of these planulae are similar to those of *P. damicornis*, but they are much less pigmented. The oral pole is much more rounded. Some planulae settled, but no survival was observed after three months. Although different substrates were provided, with different light intensities, these larvae seem not to have found the conditions they need to develop. It would be useful if we could obtain a greater number of planulae by using a larger colony, to test different conditions of settlement.

Seriatopora hystrix

Known as a brooder, this species retained our attention because of the spontaneous appearance of several colonies (< 10) on the walls of a storage tank at Oceanopolis, in 2003.

In February and March 2007 between the third and the 6th day after the new moon, the colony studied produced numerous spherical planulae with a diameter of between 150 and 500 µm and rotating. None of these larvae settled. It is probable that these planulae were not released at a stage at which they could metamorphose rapidly; this may have been caused by stress to the parent colony arising from the manipulations.

Finally on 30 March 2007 this colony released a single planula of elongated form, moving actively (size 1,300 µm) which did not settle.

***Porites* sp.**

Unlike the other species studied, this specimen seems to have responded badly to the repeated daily manipulations over two months. This resulted in a partial expulsion of zooxanthellae. We obtained two planulae with this species, in February 2007, six days after the new moon. These ovoid, mobile planulae measured about 800 µm. Only one of these larvae settled, but then disappeared after about ten days. In March 2007, four larger planulae were released on the tenth day after the new moon. They move mainly on the surface and have very little pigment (size 1,800 µm). Three of them settled, but no survival was observed after three months.

Stylophora pistillata

Four planulae were released by this species in February 2007, four and five days after the new moon. These larvae, similar to those of *P. damicornis*, seem to adhere rapidly to any substrate that they encounter. However, only one settled, two hours after having been collected. It disappeared nine days later.

Euphyllia paradivisa

Ten mobile planulae in total were released during the study. Five planulae settled, but no survival was observed after three months. Their development seems much slower than for *F. fragum* and *P. damicornis*. Although very few planulae were released, it is interesting to note that half of them settled.

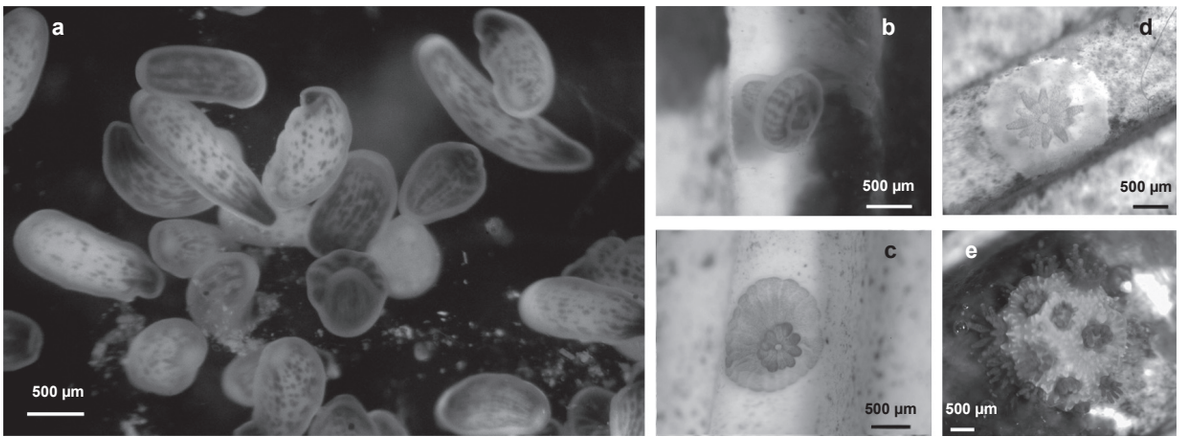

Figure 3: Pocillopora damicornis:
3a: planulae, 3b: settlement, 3c: 2 days primary polyp, 3d: 5 days primary polyp, 3e: 4 weeks colony.

Stylocoeniella guentheri

This species is easily distinguishable from *Porites* spp. by the presence of prominent spines between the calices (Veron, 2000). Until now, *S. guentheri* has not been classified as a brooder (Baird, pers. com.).

Approximatively 30 planulae were released by the colony studied in February and March 2007, two of them settled. No survival was observed after three months. Numerous spherical planulae at intermediate stages have also been observed.

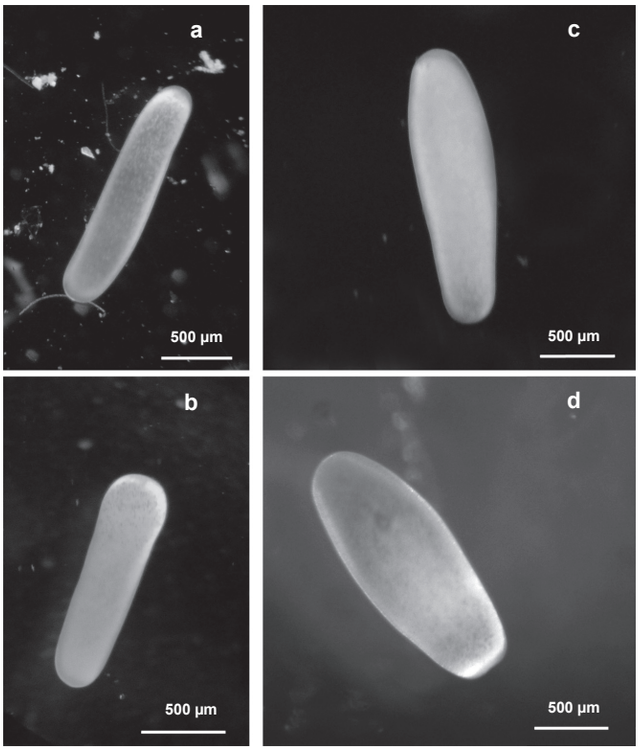

Figure 4: planula:
4a: Pocillopora verrucosa*, 4b:* Seriatopora hystrix*, 4c:* Porites *sp., 4d:* Stylophora pistillata.

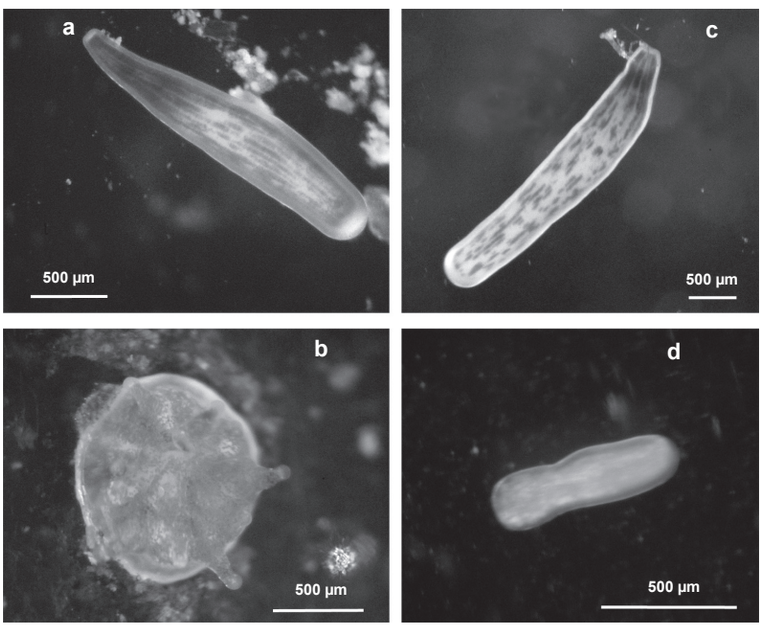

Figure 5:
5a: Euphyllia paradivisa*: planula, 5b: 4 weeks primary polyp,*
5c: Stylocoeniella guentheri*: planula,*
5d: Pavona cactus*: planula.*

Pavona cactus

To our knowledge, this species was also not considered to be a brooder. We began to study this species in March 2007. The release of planulae was first manifested by the presence of spherical intermediate stages, then by the release of mobile planulae beginning on the second day after the new moon. None of the larvae settled.

RELEASE CYCLES OF PLANULAE

The graph below (Figure 6) shows the release of planulae and early planulae for the species under study. While the release cycles of *F. fragum* and *P. damicornis* are clearly identifiable, the same cannot be said for the other species, perhaps because of the small number of planulae released. However it can be observed that the main period of release is around the eighth day after the new moon in February and March 2007.

DISCUSSION

All the selected species have shown some planulation activity during this study. This demonstrates that planulation of brooding coral species in captivity is far to be restricted to a few species like *Favia fragum* and *Pocillopora damicornis*. However, except those two species, the quantity of released planulae was always very low during this experiment. This low activity could be species-specific, as some corals are known to be both brooders and broadcast spawners. The brooding activity may be marginal for such species.

Several other factors can explain this low activity:

- Although many brooders have lunar cycles (*P. damicornis, F. fragum*), or no cycle at all (*Agaricia humilis*), some species may have annual cycles, with planulae release peaks which did not coincide with this experiment. This could be the case of *Seriatopora hystrix* (Borneman, pers. com.).
- For practical reasons, size of colonies was limited in this experiment to a maximum diameter of 15 cm. It might be possible that sexual maturity is not reached at this size for some species of this experiment.
- Sexual reproduction requires a lot of energy from the polyp. This reproduction energy budget may be not reached in captivity conditions for some species.
- Population structure within the aquariums may also be important. Density of colonies from the same

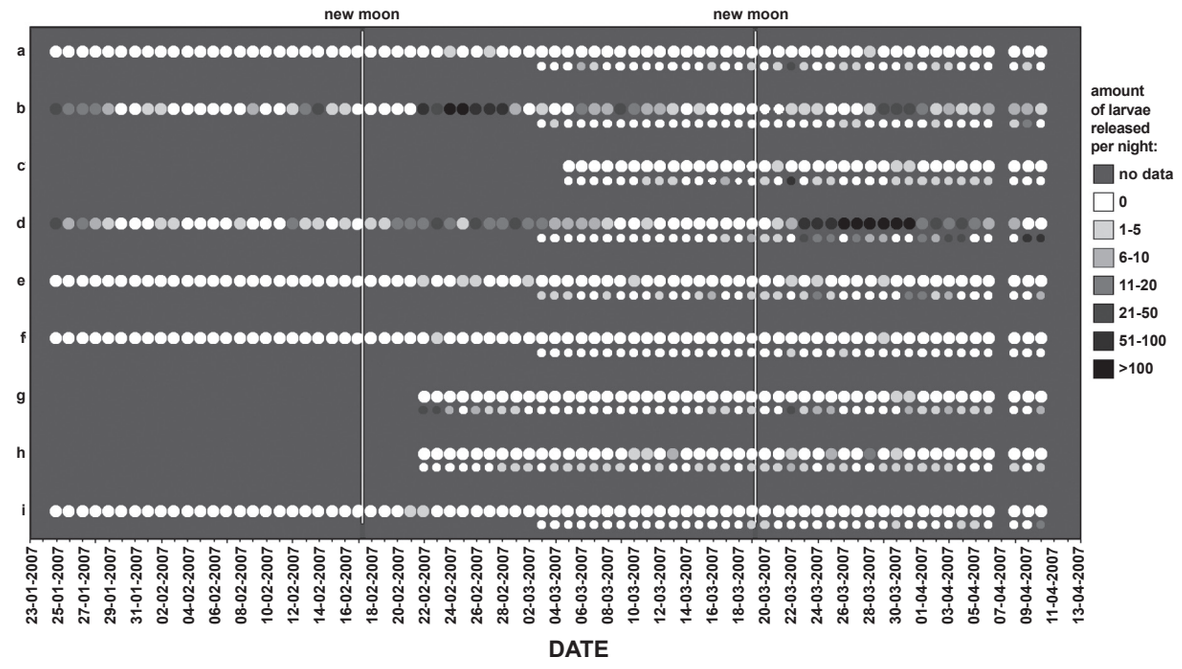

Figure 6: Planulae release cycles (big dots: planulae, small dots: early planulae stages):
a: Euphyllia paradivisa*, b:* Favia fragum*, c:* Pavona cactus*, d:* Pocillopora damicornis*, e:* Pocillopora verrucosa*, f:* Porites *sp., g:* Seriatopora hystrix*, h:* Stylocoeniella guentheri*, i:* Stylophora pistillata*.*

- species may be critical to ensure fertilization of gametes. In the wild, a distance of two meters between parents has been determined as the maximum in *Agaricia humilis* to ensure sperm transfer and internal fertilization (Morse *et al*., 1996).

Aside from the case of *P. damicornis* and *F. fragum*, no survival of primary polyps was observed after three months in this experiment. The very low number of settlers can partly explain those results, as mortality is known to be significant during the first months of life of the primary polyps. This mortality is due to environmental factors, as well as competition for space with other species, and predation. Field studies have shown that in *Litophyton arboreum*, of an estimated 1-3 x 10^6 larvae released only one established as a young colony (Gateno *et al*., 1998). It remains however obvious that our maintenance conditions for primary polyps have to be improved to ensure better survival.

Sexual reproduction of brooders will probably prove in the future to be an interesting tool for the sustainable management of *ex-situ* populations. Broodstock management and reproduction techniques have however to be improved in order to achieve good results.

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Borneman, E.H., 2007, University of Houston, Department of Biology, Science and Research Bldg II, 4800 Calhoun Houston, TX 77204, USA.